

CLAIMS

1. A method of removing more quickly non-cyclic adenine nucleotides consisting of endogenous ATP, ADP and AMP, and endogenous glucose-6-phosphate in a biological sample which comprises treating said sample with effective amounts of apyrase, alkaline phosphatase and adenosine deaminase to remove said non-cyclic adenine nucleotide and glucose-6-phosphate.

2. A method of determining cAMP content or an adenylate cyclase activity in a biological sample comprising the following steps:

Cleaning Reaction: combining a biological sample with effective amounts of apyrase, alkaline phosphatase and adenosine deaminase to remove non-cyclic adenine nucleotides consisting of endogenous ATP, ADP and AMP, and endogenous glucose-6-phosphate;

Converting Reaction: enzymatically converting cAMP in the biological sample into AMP; and

Detecting Reaction: determining an amount of AMP without the use of radioactive agents.

3. The method according to claim 2 wherein, further comprising, in said Cleaning Reaction, combining said

103 biological sample with effective amounts of glucose oxydase,
glycogen phosphorylase and alkaline phosphatase so as to
enzymatically remove endogenous glycogen from said
biological sample.

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102 4. The method according to claim 2 wherein said
Converting Reaction is carried out by combining said
biological sample with an effective amount of
phosphodiesterase.

5. The method according to claim 2 wherein an enzyme
used in said Converting Reaction of cAMP into AMP is
deactivated by a chelating agent after conversion to AMP.

15 6. The method according to claim 5 wherein said
chelating agent is EDTA.

20 7. The method according to claim 2 wherein said
Detecting Reaction comprises conversion from glycogen to
glucose-1-phosphate by contacting glycogen phosphorylase
with glycogen in the presence of inorganic phosphoric acid
added to said sample and said conversion is activated in
vitro by said AMP.

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8. The method according to claim 7 wherein said

Detecting Reaction further comprises combining said sample
 with an effective amount of phosphoglucomutase to convert
 glucose-1-phosphate into glucose-6-phosphate and then
 combining said sample with effective amounts of glucose-6-
 5 phosphate dehydrogenase to convert glucose-6-phosphate into
 6-phosphogluconolactone and NADP^+ so as to convert glucose-
 1-phosphate into 6-phosphogluconolactone and NADPH.

9. The method according to claim 8 wherein said
 10 Detecting Reaction further comprises heating up said sample
 in the presence of water to convert 6-phosphoglucono-
 lactone into 6-phosphogluconate and then combining the
 sample with an effective amount of NADP^+ to convert 6-
 phosphogluconate into ribulose-5-phosphate and NADPH.

10. A method of determining cAMP content or an
 adenylate cyclase activity in a biological sample
 comprising the following steps:

20 Cleaning Reaction: combining a biological sample with
 effective amounts of apyrase, alkaline phosphatase and
 adenosine deaminase to enzymatically remove endogenous non-
 cyclic adenine nucleotides other than cAMP, and endogenous
 glucose-6-phosphate;

25 Converting Reaction: enzymatically converting cAMP in
 the biological sample into AMP; and

Detecting Reaction: enzymatically converting ATP into fructose-6-phosphate which is then enzymatically converted into 6-phosphogluconolactone and NADPH and determining a concentration of NADPH without the use of radioactive agents.

11. The method according to claim 10 further comprising, in said cleaning reaction, combining said biological sample with effective amounts of glucose oxydase, glycogen phosphorylase and alkaline phosphatase so as to enzymatically remove endogenous glycogen from said biological sample.

12. The method according to claims 10 or 11 wherein said Detecting Reaction comprises, after enzymatically converting fructose-6-phosphate into 6-phosphogluconolactone and NADPH, further heating the reaction mixture and then adding 6-phosphogluconate dehydrogenase and NADP^+ so as to convert said 6-phosphogluconolactone into ribulose-5-phosphate and NADPH.

13. The method according to claim 10 wherein in said Cleaning Reaction, endogenous non-cyclic adenine nucleotides other than cAMP is one or more of ATP, ADP, AMP and a mixture thereof.

14. The method according to claim 10 wherein, in said
Converting Reaction, the conversion of cATP into ATP is
carried out by a combination of effective amounts of
5 phosphodiesterase, myokinase and pyruvate kinase.

15. The method according to claim 10 wherein, in said
Detecting Reaction, the conversion of ATP into fructose-6-
phosphate is carried out by a combination of hexokinase and
pyruvate kinase.

16. The method according to claim 10 wherein, in said
Detecting Reaction, the conversion of fructose-6-phosphate
into 6-phosphogluconolactone and NADPH is carried out by
combination of phosphoglucose isomerase and glucose-6-
15 phosphate dehydrogenase.

17. The method according to claim 10 wherein, in said
detecting reaction, an enzyme for conversion of ATP into
20 fructose-6-phosphate is deactivated by a chelating agent
after conversion of non-cyclic adenine nucleotides.

18. The method according to claim 17 wherein said
chelating agent is EDTA.

20. The method according to claims 1, 2 or 10 wherein
5 said biological sample is a physiological fluid.

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(22). A kit for determining cAMP content or an adenylate cyclase activity in a biological sample which comprises:

5 (1) a vial for cleaning Reaction comprising effective amounts of apyrase, alkaline phosphatase and adenosine deaminase to enzymatically remove endogenous non-cyclic adenine nucleotides other than cAMP, and endogenous glucose-6-phosphate in a biological sample;

10 (2) a vial for Converting Reaction comprising effective amounts of phosphodiesterase, ATP, myokinase, phosphoenolpyruvic acid and pyruvate kinase to enzymatically convert cAMP in the biological sample into ATP; and

15 (3) a vial for Detecting Reaction comprising fructose and hexokinase to convert ATP into fructose-6-phosphate; phosphoglucose isomerase, glucose-6-phosphate dehydrogenase and NADP⁺ to convert fructose-6-phosphate into 6-phosphogluconolactone and NADPH.

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